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Overlapping nongenomic and genomic actions of thyroid hormone and steroids

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The genomic actions of thyroid hormone and steroids depend upon primary interactions of the hormones with their specific nuclear receptor proteins. Formation of nuclear co-activator or co-repressor complexes involving the liganded receptors subsequently result in transcriptional events—either activation or suppression—at genes that are specific targets of thyroid hormone or steroids. Nongenomic actions of thyroid hormone and steroids are in contrast initiated at binding sites on the plasma membrane or in cytoplasm or organelles and do not primarily require formation of intranuclear receptor protein-hormone complexes. Importantly, hormonal actions that begin nongenomically outside the nucleus often culminate in changes in nuclear transcriptional events that are regulated by both traditional intranuclear receptors as well as other nuclear transcription factors. In the case of thyroid hormone, the extranuclear receptor can be the classical “nuclear” thyroid receptor (TR), a TR isoform, or integrin $\alpha\beta 3$. In the case of steroid hormones, the membrane receptor is usually, but not always, the classical “nuclear” steroid receptor. This concept defines the paradigm of overlapping nongenomic and genomic hormone mechanisms of action. Here we review some examples of how extranuclear signaling by thyroid hormone and by estrogens and androgens modulates intranuclear hormone signaling to regulate a number of vital biological processes both in normal physiology and in cancer progression. We also point out that nongenomic actions of thyroid hormone may mimic effects of estrogen in certain tumors.

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Introduction

Thyroid hormone and steroids have been viewed as having two distinct signaling pathways: the nongenomic transcription-independent pathway that occurs outside the nucleus and the genomic transcription-dependent pathway that occurs inside the nucleus. In the past decade, however, thyroid hormones and steroid hormones both have been found to exploit interfaces or interactions of both their genomic and nongenomic mechanisms of actions, suggesting that genomic and nongenomic signaling are not always parallel processes, but are very often sequential. In some instances, thyroid and steroid hormone signaling appear to overlap. For example, nongenomic thyroid hormone signaling can activate the nuclear estrogen receptor (ER α) in breast cancer [1] and lung cancer cells [2], and ER α expression by thyroid carcinoma has been claimed to alter course of the cancer [3].

In this review, we examine separately the overlaps of nongenomic and genomic actions of thyroid hormone and of steroids as well as the overlaps or interfaces of certain thyroid hormone and steroid actions. We emphasize possible clinical consequences of these relationships.

Overlapping genomic and nongenomic actions of thyroid hormone

The mechanism of genomic actions of thyroid hormone is well defined and has been extensively reviewed [4,5]. It requires binding by nuclear thyroid hormone receptor (TR) proteins of 3,5,3'-triiodo-L-thyronine (T₃) and the formation of transcriptionally active complexes with nuclear co-activator proteins such as p300 and steroid receptor co-activator-1 (SRC-1) [6]. The complexes bind to promoter regions of thyroid hormone-responsive genes to promote mRNA transcription. The transcripts are then translated into gene products (proteins) in endoplasmic reticulum.

Nongenomic, or transcription-independent, actions of thyroid hormone are initiated at a specific receptor on a cell surface protein (integrin α v β 3) [7] or at truncated isoforms of nuclear receptor TR α at the plasma membrane or in cytoplasm [4,8] (see below) [8]. The hormone also acts nongenomically on mitochondria via another TR isoform [9]. When signaling through α v β 3, thyroid hormone can act locally within the plasma membrane at transporter systems [10], can be extracellular—manifested at vascular growth factor receptors adjacent to the integrin [11]—or can be intracellular. Intracellular signals include regulation from integrin α v β 3 of trafficking of specific proteins from cytoplasm into the nuclear compartment [12], or activation of signal transducing kinases—e.g., mitogen-activated protein kinase (MAPK, or extracellular regulated kinase 1/2, ERK1/2) and Akt—whose downstream targets include nucleoproteins [13] and transcription factors that regulate expression of genes encoding for important regulatory proteins such as hypoxia-inducible factor-1 α (HIF-1 α) [14], basic fibroblast growth factor (*bFGF*; *FGF2*) [15], matrix metalloproteinase-9 (*MMP-9*) [16] and certain oncogenes [17] or proto-oncogenes [18]. Importantly, these thyroid hormone-mediated effects are initiated at the membrane and are then transduced into nuclear events, all independent of classical thyroid hormone receptors.

It is also important to note that the affinity of the thyroid hormone receptor on α v β 3 is significantly higher for T₄ than for T₃ [19], in contrast to hormone affinities at TR [4]. A TR α isoform (TR Δ α 1) outside the nucleus is involved in maintenance of the actin cytoskeleton by T₄ and reverse T₃ [4], and the p30 TR α 1 isoform has been identified in the plasma membrane, from which T₃ may regulate proliferation of nonmalignant cells via ERK and Akt [8]; however, these TR isoforms that may be found in cytoplasm or at the plasma membrane share no structural homologies with the hormone receptor on the integrin and are unrelated to the transcriptional events described above.

Overlapping nongenomic and genomic actions of thyroid hormone are also apparent between integrins and classical thyroid hormone receptor. For example, thyroid hormone binding to α v β 3 at the membrane regulates TR β 1 shuttling from the cytoplasm to the nucleus [12]. T₄ is bound to the S2 domain of TR β 1 that recognizes both T₄ and T₃ [14]. This process requires complexing in the cytoplasm of MAPK and TR β 1, after which TR is phosphorylated in the course of its nuclear importation. Pharmacologic inhibition of mitogen-activated protein kinase kinase (MEK) prevents nuclear uptake of both TR β 1 and MAPK. At the S1 domain of the integrin, only T₃ is a ligand and it induces cytoplasm-to-nucleus transfer of TR α 1 [14]. These observations indicate discrete control at α v β 3 for the nuclear uptake of intact TR α 1 and TR β 1—which is desirable in light of the separate functions of these two nuclear receptors [4].

An additional overlap of nongenomic and genomic mechanisms in thyroid hormone action is modulation of expression of the *TRβ1* gene itself. This became apparent when T_4 was shown to mediate internalization of $\alpha v\beta 3$ [20]. T_4 is not transported into the cell with the internalized integrin and, within the cell, the αv and $\beta 3$ monomers have disparate fates. The phosphorylated αv monomer, but not $\beta 3$, is recovered in the nucleus in thyroid hormone-treated cells in a monomer-MAPK-p300 complex. The complex binds to the promoter region of a panel of genes, including *TRβ1*, *ERα* and cyclooxygenase-2 (*COX-2*) [21] and increases expression of each of these genes. Thus, this nongenomic process of $\alpha v\beta 3$ internalization in response to T_4 supports genomic actions of T_3 by increasing availability of nuclear TR. In addition, nongenomic T_4 action at the integrin overlaps genomic effects of estrogen by increasing availability of nuclear *ERα*. As mentioned above, nuclear *ERα* is also a target of activation by thyroid hormone [1]. With regard to *COX-2*, its expression is widely regarded as a biomarker of aggressiveness in a variety of tumors. However, a stilbene, resveratrol, causes nuclear accumulation of *COX-2* protein that initiates a process of p53-dependent apoptosis in cancer cells [22]. Thyroid hormone blocks this action of resveratrol [22,23], supporting the role of the hormone in tumor cells as an anti-apoptotic agent [22,24]. Thus, the thyroid hormone receptor on $\alpha v\beta 3$ directs overlapping nongenomic and genomic actions of thyroid hormone, may serve to modulate genomic actions of estrogen, and is a contributor to regulation of the complex process of apoptosis.

In addition to regulating *TRβ1* expression, T_4 via $\alpha v\beta 3$ regulates post-translational modifications of *TRβ1*. For example, T_4 triggers MAPK-dependent phosphorylation of *TRβ1* within 10–20 min of exposure of cells to the hormone [25]. Ser-142 of TR is the target of MAPK-dependent phosphorylation, and a MAPK docking site has been identified on the thyroid hormone receptor [26]. Specific serine phosphorylation of *TRβ1* causes shedding by the receptor of corepressor proteins (SMRT) and recruitment of co-activators, e.g., p300, that contribute to the transcriptional activity of the hormone-liganded receptor. Acting at the plasma membrane, presumptively via $\alpha v\beta 3$, T_4 also regulates the phosphorylation of *ERα* [1] (see below).

Besides regulating TR phosphorylation, T_4 also nongenomically induces acetylation of the thyroid hormone receptor [6]. The acetylation step is carried out by SRC-1 and is blocked by tetrac, acting at $\alpha v\beta 3$. Acetylation of the receptor is another intranuclear event that prepares TR for recruitment of a nuclear co-activator such as p300.

Certain of the foregoing observations have led to recognition of overlapping cellular effects of thyroid hormone and estrogen and of their genomic and nongenomic mechanisms of action. For example, T_4 can, via $\alpha v\beta 3$, cause proliferation of *ERα*-expressing breast cancer (MCF-7) cells [1] and lung cancer cells [2]. The proliferative effect is *ER*-requiring and occurs in the complete absence of estrogen. Thyroid cancer cells may express *ERα* and $-\beta$ [3,27–29]. Estradiol may support *in vitro* proliferation of such cells, and *ERα* appears to be involved in growth of papillary thyroid cancer [28,29]. The progesterone receptor may also be expressed by thyroid carcinoma cells [30]. It has not been determined whether thyroid hormone can act via *ER* to stimulate proliferation of thyroid cancer cells, but integrin $\alpha v\beta 3$ does mediate a proliferative effect of T_4 on differentiated thyroid cancer cells [24].

A number of these actions of thyroid hormone are summarized schematically in Fig. 1.

Possible clinical consequences of the overlap of genomic and nongenomic actions of thyroid hormone

Acting via $\alpha v\beta 3$ at the plasma membrane, T_4 may influence genomic events within the cell. Until this pathway was recognized, T_4 was seen almost exclusively as a prohormone and source of T_3 for control of thyroid hormone actions that were genomic. Effects of T_4 on the state of cellular actin [31,32] and on plasma membrane ion pump activity in enucleate cells, such as erythrocytes [10], had been reported but did not involve gene expression. Because T_4 is now seen to be capable of modulating genomic events, the use of T_4 clinically in mild excess to suppress endogenous pituitary thyrotropin (TSH) may have genomic consequences, such as support of tumor cell proliferation [33] or expression of *HIF-1α* or of *ERα* in tumor cells in which these genes are relevant to survival or proliferation.

In the patient with subclinical hypothyroidism and active cancer, the question of how aggressive to be with T_4 replacement may be asked. Subclinical hypothyroidism induced by tyrosine kinase inhibitor treatment of renal cell carcinoma patients is associated with a favorable response to TKI

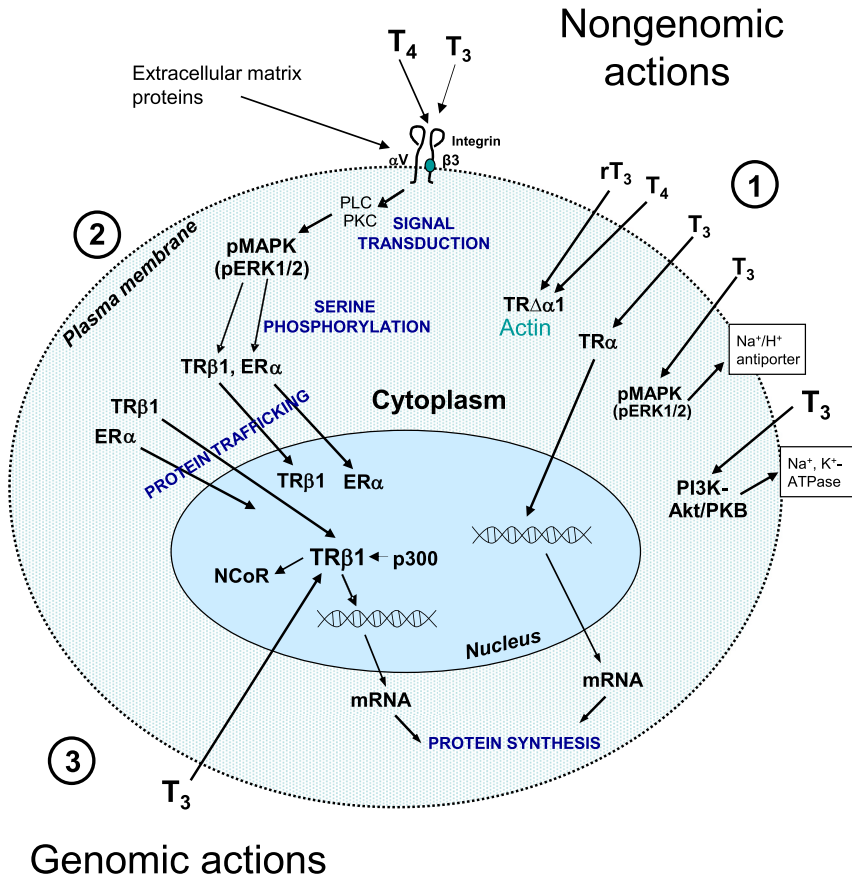


Fig. 1. Schematic of selected overlapping nongenomic and genomic actions of thyroid hormone. Nongenomic actions of T₄ and T₃ are shown to begin at the hormone receptor on heterodimeric integrin αVβ3 at the top of the figure. Actions labeled **1** indicate that from the integrin receptor the hormone can regulate the state of the actin cytoskeleton via a mechanism involving a TR_α isoform. From αVβ3 the hormone can also drive intact cytoplasmic TR_α1 (TR_α in the figure) into the nuclear compartment. T₃ acts locally in the plasma membrane to modulate activity of the plasma membrane Na⁺/H⁺ exchanger, but the hormone may act genomically to cause transcription of the exchanger gene. Similarly, T₃ acts locally to increase function of plasma membrane Na⁺, K⁺-ATPase via phosphatidylinositol 3-kinase (PI3K/Akt/PKB), but the hormone also genomically affects transcription of the sodium pump gene. In actions labeled **2**, thyroid hormone nongenomically drives cytoplasmic TRβ1 and ERα into the cell nucleus, each in a complex with activated MAPK (pMAPK; pERK1/2). Nuclear TR is partially readied for transcriptional activity—i.e., prepared to act genomically—when, in response to nongenomic signaling from hormone at αVβ3, it sheds corepressors and attracts coactivator proteins and is able to bind nuclear T₃. Actions labeled **3** are traditional genomic actions of T₃, in which the hormone gains access to the nucleus and is bound by TR to complete generation of transcriptionally active receptor-hormone-coactivator complexes.

therapy [34,35]. In a series of patients with endstage solid tumors of various origins, Herbergs and coworkers have improved survival by reducing circulating endogenous T₄ levels with low-dose thiourea administration and exogenous T₃, maintaining clinical euthyroidism exclusively with T₃ [36]. Medically induced subclinical hypothyroidism in a prospective study prolonged survival in endstage glioblastoma patients [37]. Such reports are consistent with actions of T₄ initiated nongenomically at αVβ3 that support complex tumor cell proliferation and pro-angiogenic activities that downstream of the integrin depend upon gene transcription but do not involve TRs. L-thyroxine (T₄) is primarily cited in these cancer cell observations because, as pointed out above, the affinity of the hormone receptor on αVβ3 favors T₄ over T₃ [19], and studies of cancer cell proliferation have shown T₃ to be active only at supraphysiologic concentrations, whereas T₄ is active at physiologic free hormone levels [2,14].

The ability of thyroid hormone to stimulate proliferation from the cell surface via ER α in breast cancer cells [1] raises the possibility that, in post-menopausal women, recurrent tumor may be expressing stimulation by endogenous thyroid hormone. Interestingly, *in vitro* studies have shown that fulvestrant will block the proliferative effect of T₄ in ER α -expressing breast tumor cells [1]. However, little clinical information is available about the utility or not of inducing euthyroid hypothyroxinemia [36] or subclinical hypothyroidism in this patient population. As noted above, a possible link may exist between endogenous estrogen and behavior of ER-expressing thyroid cancer.

Practice points

Acting nongenomically at the cell surface, L-T₄ can support cancer cell proliferation and tumor-related angiogenesis.

- In the patient with endstage cancer and euthyroidism, a therapeutic goal to be considered is reduction in circulating T₄ (euthyroid hypothyroxinemia) with low-dose methimazole and L-T₃ administration.
- In the postmenopausal patient with an ER α -expressing cancer of breast, ovary or lung, endogenous T₄ may act via integrin $\alpha\beta$ 3 to mimic, downstream, the action of estrogen at the estrogen receptor. Euthyroid hypothyroxinemia is an option.

Research agenda

- Develop and clinically test pharmacologic inhibitors of the thyroid hormone receptor on integrin $\alpha\beta$ 3 for management of a) advanced cancer or b) nonmalignant states in which uncontrolled angiogenesis contributes to disease expression.
- Extend evaluation of induced euthyroid hypothyroxinemia in cancer to *prospective* trials in patients with endstage cancer.

Overlapping genomic and nongenomic actions of steroid hormones

Estrogen

One of the earliest reported examples of rapid, extranuclear signaling by estradiol was by Clara Szego and June Davis in 1967. Ovariectomized rats were treated with intravenous estradiol, which led to a very rapid (within 15 s) upregulation of uterine cAMP [38]. These observations suggested that estrogen might be triggering rapid activation of a G protein within the uterine tissue, and that this in turn might regulate important estrogen-mediated physiologic responses. While Szego, Davis, and others continued to study this phenomenon for several decades, it was not until the late 1990s and 2000s that extranuclear steroid signaling became an accepted concept within the general steroid signaling community. Part of this acceptance came from the recognition that steroids such as estrogen promote rapid phosphorylation and then recruitment of steroid receptor coregulators to intranuclear transcriptional signaling complexes, thus indicating that rapid kinase signaling must be not only associated with, but likely required, for normal transcriptional signaling. Accordingly, several groups have demonstrated rapid (within minutes) estrogen-triggered G protein and kinase activation in a myriad of cell types including but not limited to breast cancer cells, uterine cells, lung cancer cells, and endothelial cells [39,40].

After confirming that rapid estrogen-triggered signals occur, the next step was to identify the receptor regulating these events. Notably, some evidence suggests that estrogen might be capable of

rapidly altering cAMP and other signals through a novel G protein-coupled receptor called “G protein-coupled estrogen receptor 1” (GPER) [41–43]. However, mice lacking GPER have relatively normal reproductive function with no obvious alterations in known estrogen-dependent processes [44]; thus the physiologic importance of GPER is still in question. In contrast, a vast body of work focusing on the classical estrogen receptors (ER α and ER β) has shown that approximately 5% of these molecules localize in the membrane (primarily full length estrogen receptor, but possibly splice variants as well) and regulate the majority of known extranuclear estrogen responses [39,40,45–50]. This membrane localization is regulated by two likely related mechanisms. First, ER α directly interacts with caveolin proteins (primarily caveolin 1) and therefore co-localizes with these molecules in lipid rafts within the membrane [50–52]. Second, Marino discovered and then Levin further characterized a palmitoylation site on ER α within a region dubbed the “E domain” that is required for membrane targeting of the protein [53–55] (see Fig. 2).

Once targeted to the membrane, ER α and ER β trigger a number of extranuclear responses. Here we will focus on ER α . As mentioned, Szego and colleagues reported rapid changes in cAMP in response to

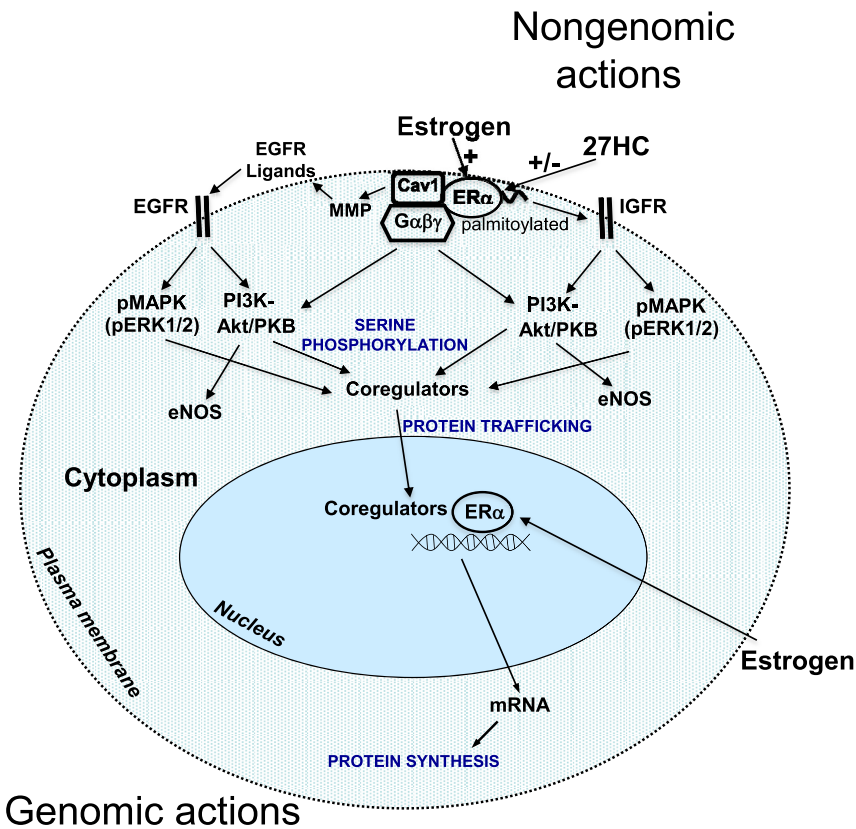


Fig. 2. Overview of estrogen receptor (ER α) signaling. ER α is localized to lipid rafts within the plasma membrane through palmitoylation as well as interactions with caveolin 1 (Cav1). Estrogen stimulation of the receptor leads to G protein activation as followed by transactivation of the EGF and IGF receptors. EGF receptor transactivation involves matrix metalloproteinase (MMP)-mediated release of EGF receptor ligands, while IGF receptor transactivation is less well understood. Both G protein signaling and receptor tyrosine kinase transactivation lead to PI3K activation, which subsequently activates eNOS in endothelial cells. In addition, PI3K and MAPK activation promote phosphorylation of coregulators, as well as perhaps direct phosphorylation of ER α , leading to increased ER α -mediated transcription and subsequent protein synthesis. Thus nongenomic and genomic ER α signaling are tightly linked. Notably 27-hydroxycholesterol (27 HC) can inhibit ER α signaling in endothelial cells to prevent blood vessel repair, but also can promote ER α signaling in other cells to increase inflammation in cardiovascular disease and to stimulate breast cancer tumor growth.

estradiol, suggesting activation of G protein signaling. Accordingly, many studies have now confirmed that estradiol rapidly modulates G protein signaling in several different cell types [39,40]. Shaul and colleagues isolated a specific region within ER α that binds directly to G proteins within the plasma membrane of endothelial cells to regulate their signaling [56–60]. Physiologically, these interactions are critical for estrogen-induced eNOS expression in endothelial cells, which in turn regulate important functions such as wound healing. Thus, one mechanism whereby estrogen may be cardioprotective in younger women is that it can improve blood vessel repair via extranuclear signaling through G proteins (Fig. 2).

In addition to regulating G protein signaling, estrogens also trigger rapid kinase signaling via membrane-localized ER α . The consensus model is that estrogen binding to membrane ER α promotes rapid activation of growth factor receptors through matrix metalloproteinase (MMP)-mediated release of membrane-associated growth factor receptors ligands. Specific growth factor receptors that have been shown to be activated include the epidermal growth factor receptor (EGFR) and the insulin-like growth factor receptor (IGFR) [61,62]. Once activated, these growth factor receptors trigger activation of Akt, ERK, and other kinase signals. Like estrogen-induced G protein signaling, estrogen-triggered activation of kinase signaling has proven to be similarly critical for wound healing [63] (Fig. 2). Also, via ER β , estrogen-induced kinase signaling appears to be an important mediator of normal cardiac function [64,65], again demonstrating the importance of extranuclear estrogen signaling in cardiovascular health. Finally, extranuclear kinase signaling, as well as G protein signaling, has been shown to be an important promoter of breast cancer cell proliferation [62,66], demonstrating another pathway (and potential target) that estrogen utilizes to promote breast cancer growth.

Estrogen is not the only ligand that can trigger rapid, transcription-independent ER α -mediated responses. Studies from the Shaul laboratory demonstrate that the cholesterol metabolite 27-hydroxycholesterol (27 HC) blocks estrogen-mediated blood vessel repair through inhibition of ER α -mediated extranuclear signaling. In addition to having antagonistic properties, 27 HC can also be an agonist for ER α , promoting worsening atherosclerosis by triggering inflammatory responses via ER α -dependent signals [67–69]. Since 27 HC and cholesterol levels are correlated, these studies suggest that hyperlipidemia may have adverse effects on cardiovascular disease through actions with ER α . Finally, 27 HC has been shown to promote breast cancer cell growth via ER α [70]. 27 HC appears to be synthesized in MCF7 breast cancer cells and is upregulated in breast cancer samples from patients, indicating that local production of 27 HC might promote progression of ER-positive breast cancer.

While both extranuclear and intranuclear estrogen signaling are known to promote important physiologic responses, how these two signals were connected to one another remained controversial until very recently. Taking advantage of the palmitoylation site that is required for membrane localization and therefore membrane signaling of ER α , two groups created an ER α knock-in mouse whereby the wild-type ER α gene was replaced with a mutated gene encoding an estrogen receptor that lacked the palmitoylation site and therefore was not capable of extranuclear signaling [71,72]. With only a few exceptions, these “nuclear-only estrogen receptor” (NOER) female mice look very much like the global ER α knockout mouse, with reduced mammary size, small uteri, infertility, and other signs of hypogonadism. These observations suggest that extranuclear ER α signaling is required for normal nuclear ER α signaling *in vivo*, and that these two processes are linear—starting with extranuclear kinase signal and ending with changes in transcription. The membrane-only estrogen receptor (MOER) knock-in mouse also looked similar to the global ER α knockout, confirming that just as ER α -mediated nuclear signaling alone is not sufficient to generate normal estrogen responses, ER α -mediated extranuclear signaling alone is also not sufficient to drive these estrogen responses [73]. In short, both extranuclear and nuclear estrogen signaling appear to be required for the majority of important physiologic estrogen functions, including fertility, breast development, and bone metabolism.

Androgen

While a tremendous amount of research from multiple laboratories has focused on the interplay between extranuclear and nuclear estrogen signaling, similar studies focused on androgen signaling have been more limited. However, many similarities between classical estrogen and androgen receptors are notable. First, the androgen receptor (AR) is palmitoylated, which regulates its localization

to the membrane [55]. Second, the AR interacts with caveolin 1 and is localized primarily in lipid rafts within the membrane [74]. Third, androgen activation mediates rapid G protein signaling via the membrane-localized AR [75–78]. Finally, androgen activation of membrane-localized AR leads to rapid transactivation of the EGF receptor via a MMP-mediated release of EGF receptor ligands, after which both Akt and MAPK pathways are activated, the latter of which is important for subsequent nuclear AR-mediated signaling [79–83] (Fig. 3).

Work over the last decade has uncovered a novel regulator of both extranuclear and nuclear androgen signaling: paxillin. Paxillin is normally described as a primarily cytoplasmic scaffolding molecule that regulates a myriad of cytoplasmic functions that include kinase signaling, integrin signaling, cytoskeletal rearrangements, and cell–cell adhesion [84–87]. However, several studies now demonstrate that paxillin plays an additional role in regulating androgen signaling both in the cytoplasm and in the nucleus [81,82,88,89]. Surprisingly, paxillin's importance in androgen signaling was first discovered in oocytes from *Xenopus leavis*. For over 50 years, steroids have been known to trigger meiotic re-entry in oocytes (often called “maturation”) [77]. Since meiosis occurs completely independent of transcription, steroid-triggered oocyte maturation is therefore an outstanding example of a

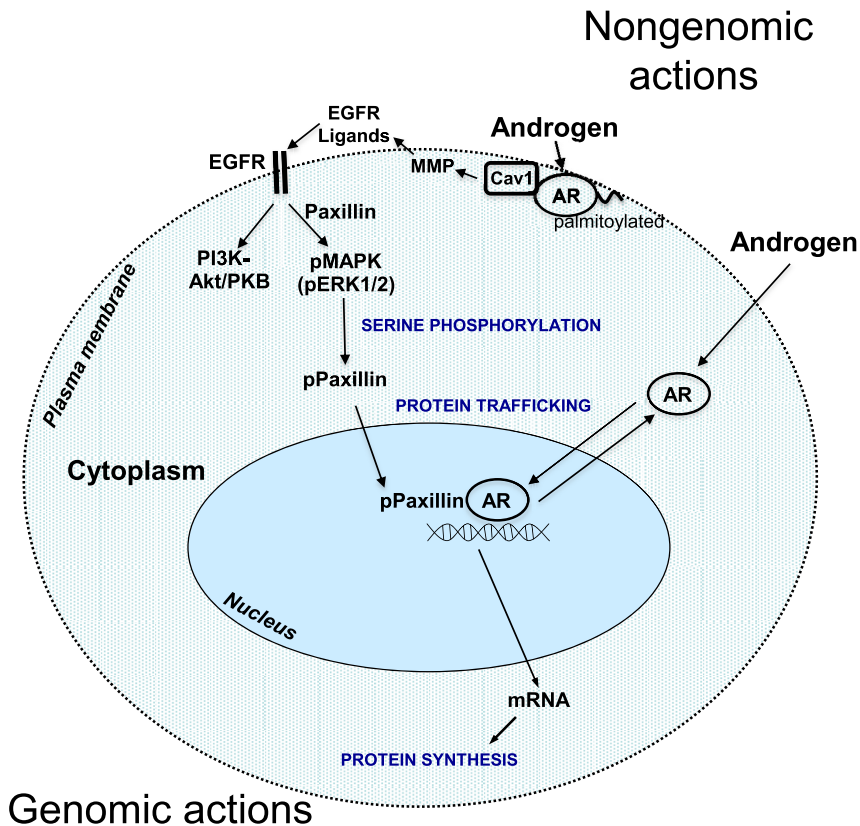


Fig. 3. Nongenomic androgen receptor (AR) signaling enhances genomic AR signaling. Androgen stimulates ARs that are localized to lipid rafts within the plasma membrane through palmitoylation as well as interactions with caveolin 1 (Cav1). AR activation at the membrane leads to matrix metalloproteinase (MMP)-mediated transactivation of the EGF receptor (EGFR) via release of EGF receptor ligands. EGF receptor signaling then activates downstream PI3K and MAPK signaling. The latter is dependent on paxillin. Once activated ERK1/2 promotes phosphorylation of paxillin on serine residues, leading to the nuclear localization of phosphopaxillin (pPaxillin). Once in the nucleus, pPaxillin retains androgen-bound AR in the nucleus, where the AR can promote gene transcription and subsequent protein synthesis. Thus paxillin is a critical regulator of both genomic and nongenomic AR signaling, and serves as a liaison between cytoplasmic and nuclear events.

physiologically relevant, purely nongenomic steroid-triggered process. Furthermore, since androgens appear to be physiologic mediators of *Xenopus laevis* oocyte maturation [90], this system serves as an excellent model for studying extranuclear androgen signaling. Using this model system, studies have shown that just as estrogens regulate G protein and kinase signaling in somatic cells, androgen-induced oocyte maturation requires rapid changes in G protein signaling as well as activation of MAPK signaling [75,78,91–93]. Notably, a key regulator of androgen-triggered kinase signaling in oocytes is paxillin because knockdown of its expression abrogates steroid-triggered kinase signaling and downstream maturation [89]. Studies in oocytes have implicated ERK-mediated phosphorylation of serine residues within paxillin as important regulators of oocytes' maturation downstream of ERK signaling because mutating these serines to alanines results in a paxillin molecule that can still mediate androgen-induced ERK activation, but is unable to mediate subsequent meiotic resumption. Thus, in essence, paxillin is both upstream and downstream of ERK signaling and plays an important biological role in regulating meiosis.

Based on the aforementioned work in frog oocytes, investigators have been able to demonstrate that paxillin is also a critical regulator of prostate cancer growth in humans [81,82]. In several human prostate cancer cell lines, paxillin is similarly found to be required for ERK signaling, which in turn regulates phosphorylation of human paxillin on serine residues similar to those present in the frog isoform. This serine phosphorylation event then promotes nuclear localization of phosphoserine-paxillin, where it interacts with nuclear AR to retain it in the nucleus and to support AR-mediated transcription of a number of endogenous prostate genes (Fig. 3). As with the frog paxillin, mutation of these serine phosphorylation targets to alanines completely abrogates androgen-mediated transcription, while androgen-induced ERK signaling still occurs normally. In the absence of phosphorylated paxillin, prostate cancer growth, migration, and invasion are markedly attenuated. Finally, paxillin expression is elevated in human prostate cancer samples, suggesting that nongenomic androgen actions may be upregulated in prostate cancer. Thus, in its capacity as a liaison between extranuclear and nuclear androgen signaling, paxillin appears to be a critical regulator of androgen-triggered prostate cancer progression and may serve as a useful marker of prostate cancer aggressiveness or perhaps even as a potential target for prostate cancer therapy.

Finally, paxillin actions are not just important for AR signaling in prostate cancer cells. Recent work demonstrates that ARs similarly use both extranuclear and nuclear pathways to regulate ovarian follicle development by both inhibiting follicle atresia and also promoting FSH-mediated follicle growth [83]. With extranuclear and nuclear androgen signaling, both of which are mediated by paxillin, follicles do not grow appropriately, and fertility is compromised. Thus, besides regulating pathologic androgen-mediated growth in prostate cancer, paxillin is also an important regulator of normal androgen-regulated physiologic processes such as female fertility.

With regard to paxillin's importance in ER α -mediated signaling, just as paxillin levels are upregulated in prostate cancer samples, paxillin levels are also upregulated in some aggressive Her2 positive breast cancers [94]. Therefore, as seen in prostate cancer, paxillin may be an important regulator of breast cancer progression. However, on a molecular level, while paxillin may be playing a small role in modulating extranuclear estrogen signaling [95], its role in regulating estrogen-mediated transcription remains unclear.

Possible clinical consequences of overlapping genomic and nongenomic actions of steroids

The clinical consequences of overlapping genomic and nongenomic actions with steroid hormones are myriad. Cardiovascular protection by estrogen appears to require both extranuclear (via G proteins and kinases) and nuclear (via transcription) ER α actions. In the example of cardiovascular disease, non-nuclear ER α may actually dominate, both in a positive way in response to estradiol and in a negative way in response to 27 HC. Through local production, both estradiol and 27 HC may use overlapping nongenomic and genomic actions of ER α to promote breast cancer growth in ER-positive tumors. Knock-in mouse models where nuclear and extranuclear ER α actions are clearly isolated from one another demonstrate that nearly all of the important known actions of estrogens in the pituitary, ovary, uterus, and mammary glands require both genomic and nongenomic ER α signaling. These studies are crucial because they are the first to use genetic mouse models to clearly show the physiologic

significance of combined nuclear and extranuclear steroid effects. Finally, with regard to androgen signaling, studies both in prostate cancer and in the ovary demonstrate the sequential nature of extranuclear and nuclear AR signaling and implicate paxillin as an important liaison between these two cellular compartments. Since paxillin expression is upregulated in prostate cancer and in some aggressive breast cancers, this molecule may serve as an important biomarker for tumor aggressiveness as well as a potential target for the treatment of hormone-dependent cancers.

Practice points

- Integration of extranuclear and nuclear steroid signaling plays a critical role in many important physiologic processes, including fertility, cardiovascular function, and growth of hormone dependent cancers.
- Cholesterol metabolites such as 27-hydroxycholesterol can signal through the estrogen receptor to worsen atherosclerosis, inhibit wound repair, and possibly promote breast cancer growth.
- Balanced androgen signaling in the ovary is critical for normal female fertility – too little or too much androgen signaling leads to reduced follicle development and ovulation.

Research agenda

- Develop novel therapies to target the integration of nuclear and extranuclear signaling in steroid-dependent cancers of the prostate and breast.
- Improve our understanding of how cholesterol metabolites regulate cardiovascular disease so that we can potentially manipulate their levels in a therapeutically advantageous manner.

Conclusions

Recent reports have demonstrated that nongenomic and genomic signaling by both thyroid hormones and steroids are inextricably linked, such that most important transcriptional changes in response to these hormones require both processes to be functioning normally. Nongenomic signaling can arise from classical “nuclear” hormone receptors on or near the cell surface and from other important cell membrane signaling molecules such as $\alpha v\beta 3$. These nongenomic signals can modulate nuclear transcription via many pathways, including nuclear localization of classical nuclear receptors or changes in phosphorylation of important nuclear signaling molecules. Understanding how genomic and nongenomic signaling work together to regulate important biological processes will help us better design novel means of regulating these processes and therefore may improve our ability to treat hormone-dependent cancers such as breast and prostate cancer, as well as other tumors such as lung cancer.

Disclosure

The authors have no conflicts of interest to disclose.

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